Does ischemic preconditioning reduce spinal cord injury because of descending thoracic aortic occlusion?

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Objective: Ischemic preconditioning has been found to protect various organs from a subsequent longer ischemic insult. We investigated whether the late phase of ischemic preconditioning reduces spinal cord injury from occlusion of the descending thoracic aorta.

Methods: Twenty-four pigs (27 to 30 kg) were randomly divided in four groups: group I (n = 4) underwent a sham operation, group II (n = 4) underwent aortic occlusion for 20 minutes, group III (n = 8) underwent aortic occlusion for 35 minutes, and group IV (n = 8) underwent aortic occlusion for 20 minutes and, 48 hours later, aortic occlusion for 35 minutes. Aortic occlusion was accomplished with two balloon occlusion catheters placed fluoroscopically at T6 to T8 above the diaphragm and at the aortic bifurcation. Neurologic evaluation was performed by an independent observer according to Tarlov’s scale (0 to 4, with 4 as normal). The lower thoracic and lumbar spinal cords were harvested at 120 hours and examined histologically with hematoxylin and eosin stain. Histologic results (number of neurons and grade of inflammation) were scored 0 to 4 (4, intact spinal cord; 0, no neurons and high inflammation) and were similarly analyzed. Results were expressed as the mean ± the standard error of the mean, and statistical analysis used the Kruskal-Wallis test.

Results: Group IV had a better neurologic outcome at 24, 48, and 120 hours in comparison with group III (P < .001), although 120 hours after the end of the experiment, the neurologic outcome in group IV was worse than at 24 hours (P = .014). The histologic changes were proportional to the neurologic test scores, with the more severe and extensive gray matter damage in the animals of group III (number of neurons, P < .001; and grade of inflammation, P < .001).

Conclusion: Ischemic preconditioning (late phase, 48 hours after the first occlusion) reduces spinal cord injury after aortic occlusion, as estimated with Tarlov’s score and histopathology. (J Vasc Surg 2003;37:426-32.)

Operations that require aortic occlusion (AOC) result in ischemia to the distal organs. The most vulnerable of these organs is the spinal cord. Neurologic injury is a result of AOC in the absence of adequate collateral flow and increases with the duration of occlusion. The reported prevalence rate of neurologic injury (paraplegia/paraparesis) from such operations ranges from 5% to 16%.1-6 Spinal cord injury is even more frequent among patients who need extent II thoracoabdominal aortic aneurysm repair, with paraplegia rates ranging from 8% to 32%.6-9

Spinal cord ischemia in the perioperative period can result from distal aortic hypotension, the interruption of critical intercostal and lumbar arteries, and thrombosis or embolism of intercostal arteries. In addition, reperfusion with oxygenated blood after ischemia could aggravate the spinal cord injury. In general, the mechanisms responsible for ischemia/reperfusion (I/R) injury are excitotoxicity, nitric oxide–mediated neuronal cell death, intracellular calcium overload, eicosanoid formation, inflammation, apoptosis, and oxygen free radical–induced damage.10,11 Numerous methods, both pharmacologic12 and mechanical,13,14 have been used to decrease the incidence of neurologic injury, but no randomized studies confirm consistent prevention of neurologic complications.

Recently, previous reports in small animal models have shown a beneficial effect of ischemic preconditioning (IPC) on spinal cord protection. IPC is the process whereby a sublethal ischemic insult enhances the tolerance of the tissue to a subsequent ischemic stress. IPC is an endoge-
nous cellular protective mechanism, which was subsequently found to be a biphasic phenomenon, with an early\textsuperscript{15} and a late\textsuperscript{16} phase of protection. We sought to investigate the effect of IPC and especially the beneficial effect of the late phase on spinal cord protection after descending thoracic AOC. In this experiment, we applied those encouraging results to a large animal model that is closer to clinical practice and offers more direct correlation to actual elective operative circumstance 2 days after patient angiography. To our knowledge, this kind of experiment has not been conducted before.

**MATERIALS AND METHODS**

**Experimental protocol.** A total of 24 pigs of either gender were used in this study. All animals weighed between 27 and 30 kg and were randomly assigned to four groups. Group I (n = 4) underwent a sham operation. Group II (n = 4) underwent AOC for 20 minutes, group III (n = 8) underwent AOC for 35 minutes, and group IV (n = 8) underwent AOC for 20 minutes and, 48 hours later, AOC for 35 minutes. All animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication No. 86-23, revised 1985), and the animal protocol was approved by the Institutional Animal Care and Use Committee of the University of Ioannina, Greece.

**Experimental preparation.** All animals were fasted for 12 hours before the procedure. The pigs were anesthetized with an intramuscular injection of azeperone (4 mg/kg) and, after 20 minutes, ketamine hydrochloride (5 to 10 mg/kg). Then, intravenous administration of general anesthesia and fluids was placed in two veins in the ears. General anesthesia consisted of thiopental sodium 2.5% in a dose 10 to 15 mg/kg, and the animals were tracheally intubated and given intravenous ketamine hydrochloride (1 to 2 mg/kg), atracurium besylate (0.4 to 0.6 mg/kg and 0.1 mg/kg every 20 to 30 minutes), fentanyl citrate (1.5 to 8 μg/kg), and gentamicin sulfate (1 mg/kg). General anesthesia was maintained with sevoflurane 1% to 4% depending on the phase of the experiment. Their lungs were ventilated with 100% oxygen, with the arterial PaO\textsubscript{2} tension maintained at more than 100 mm Hg, PaCO\textsubscript{2} 35 to 45 mm Hg, and pH was normal, as confirmed with arterial blood gas analysis. All animals were placed on a warming blanket, and rectal temperature was maintained at 35.9°C to 36.7°C. The electrocardiogram was continuously recorded with needle electrodes.

With sterile conditions, the right femoral artery, left jugular vein, and left common carotid artery were isolated, and after anticoagulation with intravenous heparin (300 IU/kg in all animals), catheters were inserted (Seldinger method). The arteries were catheterized with 11F catheters to provide continuous monitoring of the arterial blood pressure (carotid artery, proximal and femoral artery, distal to AOC). The jugular vein was catheterized with a 9F catheter. A 7F pulmonary artery catheter was advanced through the external jugular vein to ensure optimal hemo-dynamic monitoring and guide fluid resuscitation during the experiment. With arterial blood pressure monitoring, with maintaining systolic blood pressure at more than 90 mm Hg, blood (7 mL/kg) was drained into a citrate bag collection to be returned during subsequent resuscitation after blood flow restoration in the occluded aorta.

In this experimental model, the AOC was performed with two aortic balloon occlusion catheters, size 9F (occlusion balloon catheters, Medi-Tech Boston Scientific, Boston, Mass), which were inserted with fluoroscopic guidance. The first was inserted through the carotid artery to the supraceliac aorta and pulled up towards the carotid artery to achieve thoracic AOC between T\textsubscript{6} and T\textsubscript{8} and be entirely above the diaphragm when inflated. The second balloon was inserted through the femoral artery to the aortic bifurcation and the common iliac arteries (to avoid collateral circulation). During AOC and 1 hour after the blood flow restoration, the heart rate, arterial blood pressure (proximal, aortic isolated segment and distal), and rectal temperature were recorded every 5 minutes. After AOC (in groups II, III, and IV), the balloons were deflated and all animals were fully resuscitated with intravenous fluids (Ringer’s lactate) and the reinfusion of autologous blood. Sodium bicarbonate (1 mEq/kg) was administered to restore acid-base status, and blood pressure was restored with phenylephrine hydrochloride (0.1 to 0.2 mg in bolus infusion during the first minutes after aortic unclamping). After 60 minutes of reperfusion, all the animals were hemodynamically stable without the need of fluid or drug administration. All the catheters were removed, and all wounds were closed in anatomic fashion. When the pig was breathing spontaneously, the trachea was extubated and another dose of gentamicin sulfate (1 mg/kg) was administered intravenously. Finally, the animals were placed in their cages for follow-up.

Animals in group I (sham operation) underwent the previous procedure, but the balloons were not inflated. In group IV, we performed two AOCs, with an interval of 48 hours between them. Before each AOC, blood was taken from the animals for the two autologous reinfusions.

**Neurologic evaluation.** At 24, 48, and 120 hours after the end of the experiment, all animals were evaluated by an independent observer according to the Tarlov scoring system.\textsuperscript{17} (T\textsubscript{0}, spastic paraplegia and no movement of the lower limbs; T\textsubscript{1}, spastic paraplegia and slight movement of the lower limbs; T\textsubscript{2}, good movement of the lower limbs but inability to stand; T\textsubscript{3}, ability to stand but inability to walk normally; and T\textsubscript{4}, complete recovery.)

**Postmortem examination.** Postmortem examination of the abdomen was performed.

**Histologic study.** The animals were killed 120 hours after the end of the experiment with an overdose injection of sodium pentobarbital, and spinal cords specimens were harvested immediately for histologic study with light microscopy. The lower thoracic and lumbar spinal cords were fixed in a 10% formalin solution for 120 hours before being set in paraffin blocks for sectioning. Three representative glass slices with 7 μm-thick sections were obtained from...
Table I. Systolic arterial blood proximal and distal

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<th>Baseline</th>
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<tr>
<td></td>
<td>Proximal SAP</td>
<td>Distal SAP</td>
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<tr>
<td>Group I</td>
<td>108 ± 4</td>
<td>113 ± 4</td>
</tr>
<tr>
<td>Group II</td>
<td>106 ± 8</td>
<td>109 ± 7</td>
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<tr>
<td>Group III</td>
<td>114 ± 6</td>
<td>112 ± 8</td>
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<tr>
<td>Group IV</td>
<td>108 ± 5</td>
<td>112 ± 5</td>
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<tr>
<td>P value</td>
<td>.111</td>
<td>.829</td>
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Temperature measured rectally. Data expressed as mean ± standard deviation. Statistical analysis with one-way analysis of variance.

AISP, Aortic isolated segment pressure.

Each animal at L3 and stained with hematoxylin and eosin. A pathologist unaware of the pig’s neurologic outcome examined each slide with light microscopy to count the total number of motor neurons in the half gray matter region. Also, the grade of inflammation was scored 0 to 4 (score 0, very high grade of inflammation with high vascularization with hyperemia; score 1, high grade of inflammation with high vascularization with hyperemia; score 2, moderate inflammation with moderate vascularization with or without hyperemia; score 3, low inflammation with moderate vascularization with or without hyperemia; and score 4, no inflammation with normal vascularization without hyperemia).

Statistical analysis. Data are presented as the mean ± the standard deviation or as the mean ± the standard error of the mean as noted in each table. Statistical evaluation was performed with one-way analysis of variance for comparison of experimental variables between groups. The difference among groups in terms of the Tarlov score and the grade of inflammation was determined with nonparametric statistical analysis with the Kruskal-Wallis test. A P value of less than .05 was considered significant as determined with SPSS 8.0 Software (SPSS, Inc, Chicago, III).

RESULTS

Hemodynamic measurements. The animals did not differ with respect to blood gas analysis. No significant difference was seen between groups II, III, and IV with regard to mean systolic arterial pressure and heart rate during the AOC and during reperfusion proximal and distal. Between group I and groups II, III, and IV, significant difference was seen in mean heart rate and mean systolic arterial pressure proximal and distal during the AOC. No significant difference was seen in aortic isolated segment pressure in groups II, III, and IV, as seen in Table I.

Neurologic outcome. The results are summarized in Table II. All animals in group I (n = 4) and group II (n = 4) had normal neurologic outcome (Tarlov score 4) at 24, 48, and 120 hours.

In group III (n = 8) at 24 hours, two animals (25%) had Tarlov score 4, five animals (62.5%) had Tarlov score 2, and one animal (12.5%) had score 1. At 48 hours, one animal (12.5%) had score 4, six animals (75%) had score 2, and one animal (12.5%) had score 4; and at 120 hours, six animals (75%) had score 2 and two animals (25%) had score 1. In group IV (n = 8) all animals had normal neurologic outcome (Tarlov score 4) at 24 and 48 hours after the first AOC of 20 minutes. The other measurements were taken after completion of the second AOC of 35 minutes (Tables II, III, and IV). At 24 hours, all animals had normal neurologic outcome (Tarlov score 4). At 48 hours, six animals (75%) had Tarlov score 4 and two animals (25%) had score 3; and at 120 hours, two animals (25%) had Tarlov score 4 and six animals (75%) had score 3. A significant difference was seen in neurologic outcome between group III and groups I, II, and IV at 24, 48, and 120 hours (P < .001).

An aggravation of mean Tarlov scores is seen in groups III and IV, comparing the results at 24 and 120 hours after the end of experiment. This aggravation is statistically significant only in group IV (P = .014, with Wilcoxon test) and not in group III (P = .083, with Wilcoxon test).

Postmortem examination. Postmortem examination of the abdomen in all 24 animals failed to show gut ischemia or necrosis with gross examination. No clinical signs of ischemia, such as blood in the stool, diarrhea, or lack of appetite, were observed.

Histologic evaluation. The results of neuron counting in the half gray matter region are shown in Table III. According to the scoring system noted previously, animals in group III had the worst score (74 ± 9 motor neurons; 1.63 ± 0.42 inflammation) in comparison with groups I (179 ± 7 motor neurons; 4.00 ± 0.00 inflammation score), II (172 ± 10 motor neurons; 4.00 ± 0.00 inflammation score), and IV (104 ± 17 motor neurons; 2.88 ± 0.28 inflammation score), and these differences were statistically significant (P < .05).

DISCUSSION

Spinal cord injury from hypoperfusion after a successful operation on the thoracic aorta remains a potentially devastating and unpredictable complication. Occlusion of the descending thoracic aorta has been shown to reduce spinal blood flow in a variety of animal models, and this effect has been documented in humans as well. Several protective strategies have been developed either to preserve the blood supply of the spinal cord or to increase the ischemic tolerance of the spinal cord. Despite various efforts, no method has totally prevented the development of paraplegia.
A brief I/R episode can provide protection against the deleterious effects of subsequent more severe I/R challenge, a phenomenon referred to as IPC. Studies regarding the time course for the protective effects of IPC have revealed two temporally and mechanistically distinct types of protection. The protective effects of a previous I/R episode become apparent within minutes after the initial insult and persist for approximately 1 to 2 hours, after which the susceptibility of the tissue to I/R returns to normal. This early and transient protection has been referred as early or acute IPC. In addition to the short-lived protection afforded with I/R pretreatments, there is also an adaptable response in the affected organ that renders it resistant to I/R injury for 24 hours or longer after the initial insult and can persist for several days. This type of IPC has been referred as late or delayed IPC.

In this study, we investigated whether the late phase of IPC phenomenon could be induced with brief occlusion of the pig descending aorta and reduced spinal cord injury. This is the first study in the literature to evaluate the late phase of IPC in pigs.

Previous reports have shown a beneficial effect of IPC against spinal cord ischemic injury. Matsuyama et al were the first to evaluate the late phase of IPC in a dog model. The IPC group had 20 minutes of descending thoracic aortic cross clamping and, 48 hours later, 60 minutes of cross clamping. The preconditioned animals had better neurologic outcome than did the untreated animals; however, they were followed for only 24 hours after ischemia. Sakurai et al showed, in a rabbit model of infrarenal AOC, that animals that underwent 10 minutes of IPC were protected from a subsequent ischemic insult of 15 minutes occurring 48 hours later. However, their study used a single neurologic examination at 7 days after the end of the experiment and did not follow the development of neurologic injury and recovery. Abraham et al evaluated the effect of late IPC in a rat model. The rats underwent 2 or 5 minutes of brief ischemia and, 48 hours later, underwent 10 minutes of descending thoracic AOC. Their study used both a four-point and a 15-point score of hind limp motor index. They concluded that there was a lesser neurologic deficit in the group with 5 minutes of IPC, especially in the group with 5 minutes of IPC, especially in the group with 5 minutes of IPC. In contrast to these studies indicating the beneficial effect of IPC, de Haan et al failed to reduce spinal cord injury in a rabbit model with 6 minutes of IPC and 26 minutes of infrarenal AOC 24 hours later.

The protection provided by IPC is dependent on the duration of ischemic pretreatment, the time window between pretreatment and subsequent insult, and the severity of the subsequent insult. Major reports have shown that paraplegia in the pig model occurs if the duration of AOC equals or exceeds 30 minutes. Therefore, the aorta was occluded 20 minutes for IPC of the spinal cord, and this duration was conformed to a sublethal insult to the spinal cord because all the animals (group II) had a completely normal neurologic outcome and no lesions in the histologic evaluation of the spinal cord (Fig 1, B). The spinal cord ischemia model was developed to produce a less than 100% incidence rate of paraplegia, as it occurs in the clinical situation. Of note is the isolated aortic segment pressure of 14 mm Hg average that indicates absence of significant flow in the 20 experiments with AOC.

Table II. Tarlov scores at 24, 48, and 120 hours after end of experiment

<table>
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<tr>
<th>Group</th>
<th>24 h</th>
<th>48 h</th>
<th>120 h</th>
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<tr>
<td>I (n = 4; sham operation)</td>
<td>4.00 ± 0.00</td>
<td>4.00 ± 0.00</td>
<td>4.00 ± 0.00</td>
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<tr>
<td>II (n = 4; 20 min AOC)</td>
<td>2.13 ± 0.23</td>
<td>2.00 ± 0.19</td>
<td>1.75 ± 0.16</td>
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<tr>
<td>III (n = 8; 35 min AOC)</td>
<td>4.00 ± 0.00</td>
<td>3.75 ± 0.16</td>
<td>3.25 ± 0.16</td>
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<tr>
<td>IV (n = 8; late IPC)</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
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Data expressed as mean ± standard error of mean. Statistical analysis with nonparametric Kruskal-Wallis test.
Balloon occlusion of the thoracic aorta, as used in these experiments, has many advantages for investigation of pathophysiologic changes after spinal cord ischemia. The surgical procedures are simple, quickly accomplished, and less invasive than aortic cross clamping through a thoracotomy. The special significance of this animal model in comparison with others is that it offers closer correlation to possible elective intervention 2 days after angiography, all performed through catheters. The balloon is extended over two intervertebral spaces (T6 to T8) into the aorta and occludes the intercostal arteries included in this segment of the aorta. This occlusion of intercostal arteries may have a beneficial effect on spinal cord perfusion by avoiding steal phenomena. However, all animals in the untreated group (group III) had a neurologic deficit. We ruled out the effect of hypothermia and hypertension because all groups of animals had similar rectal temperatures and blood pressure.

The difference in the distribution of the lost neurons in the ischemic spinal cord is of interest. Neurons located in central areas of the gray matter, an area known to sustain a more severe ischemic injury, may die first of severe membrane failure and necrosis. In accordance with this theory, the loss of neurons in the untreated group is more extensive in the central areas of the gray matter, and this finding may validate the adequacy of our experimental model. Other studies have shown the possible relation between leukocytes and pathogenesis and extension of ischemic injury. Inflammatory cells are responsible for ischemic neuronal injury because these cells are found in the motor injured area and direct phagocyte effect or the secretion of toxins from these cells have cytotoxic effects. Also, apoptosis has been shown to be an important mode of cellular death in the ischemic spinal cord. The partial preservation of neurons and the reduction of inflammation in the IPC group suggest a role for IPC in the protection from activation of neutrophil and apoptosis after reversible spinal cord ischemia.

Histologic assessment in our experiment indicates that 59% of neurons in the untreated group III were lost, the grade of inflammation worsened by 59%, and these findings independently show that the neurologic deficit, as assessed with Tarlov score (reduction, 56%), was valid. Moreover, in the treated IPC group IV, 42% of neurons were lost, the

<table>
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<th>No. of neurons</th>
<th>Grade of inflammation</th>
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<tr>
<td>Group I (n = 4; sham operation)</td>
<td>179 ± 7</td>
</tr>
<tr>
<td>Group II (n = 4; 20 min AOC)</td>
<td>172 ± 10</td>
</tr>
<tr>
<td>Group III (n = 8; 35 min AOC)</td>
<td>74 ± 9</td>
</tr>
<tr>
<td>Group IV (n = 8; late IPC)</td>
<td>104 ± 17</td>
</tr>
<tr>
<td>P-value</td>
<td>&lt;.001</td>
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</table>

Data expressed as mean ± standard deviation (number of motor neurons) and as mean ± standard error of mean (grade of inflammation). Statistical analysis with one-way analysis of variance for number of motor neurons and with nonparametric Kruskal-Wallis test for grade of inflammation.

Fig 1. Representative photographs of lumbar spinal cord sections stained with hematoxylin and eosin. No neuronal damage to any motor neuron cells was found in animals of group I with sham operation (A) and of group II of 20 minutes AOC (B). At 120 hours after 35 minutes of ischemia, about 60% of motor neuron cells in gray matter were lost in untreated group III, and inflammation is obvious (C). At 120 hours after 35 minutes of ischemia in group of late IPC, about 40% of motor neuron cells were lost, and grade of inflammation is lower (D). Original magnification, ×330.
grade of inflammation worsened by 38%, and the Tarlov score at 120 hours was reduced only by 19%. Different results might have been obtained if more sophisticated testing had been used, as in the study by Abraham et al. A Tarlov score of 3 means that the animal is able to stand and walk (IPC group IV), and a Tarlov score of 2 (untreated group III) means that the animal is unable to stand, and this represents a fundamental clinical difference. All these observations became apparent in our study because the animals were followed for 120 hours. Although all the animals in group III (untreated) and untreated animals (group III) means that the animal is unable to stand, and this testing had been used, as in the study by Abraham et al. A

Table IV. Tarlov scores at 24, 48, and 120 hours, number of neurons in half gray matter, and grade of inflammation in treated (late IPC) and untreated (group III)

<table>
<thead>
<tr>
<th>Tarlov 24 h</th>
<th>Tarlov 48 h</th>
<th>Tarlov 120 h</th>
<th>No. of neurons</th>
<th>Grade of inflammation</th>
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<tr>
<td>Group III (n = 8; 35 min AOC)</td>
<td>2.13 ± 0.23</td>
<td>2.00 ± 0.19</td>
<td>1.75 ± 0.16</td>
<td>74 ± 9</td>
</tr>
<tr>
<td>Group IV (n = 8; late IPC)</td>
<td>4.00 ± 0.00</td>
<td>3.75 ± 0.16</td>
<td>3.25 ± 0.16</td>
<td>104 ± 17</td>
</tr>
<tr>
<td>P value</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
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<td>.001</td>
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Data expressed as mean ± standard error of mean, except for number of neurons (mean ± standard deviation). Statistical analysis with nonparametric Mann-Whitney U test, except for number of neurons comparison (independent samples t test).

We thank Mr Dimitrios Athanasous for his excellent technical assistance in preparing this experimental model.

REFERENCES


